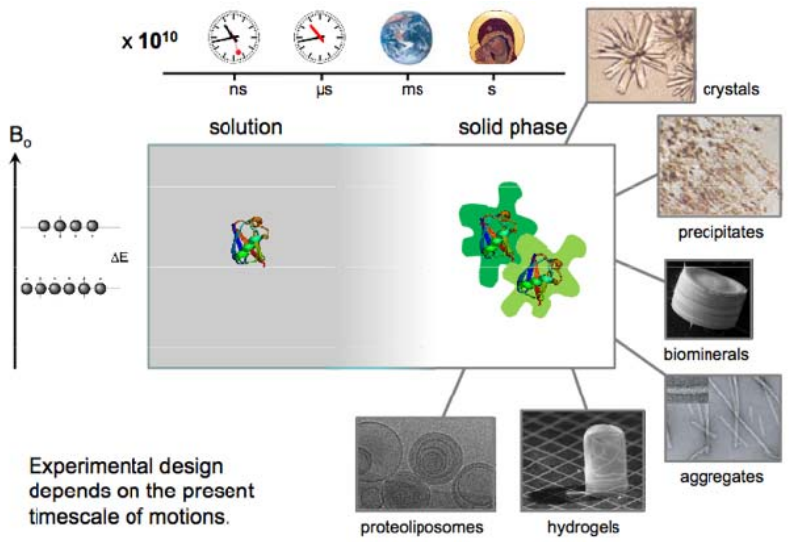
Introduction to solid-state NMR on biomolecules

Renafobis Oleron 2016
May 25th 2016

Marie Renault

Application fields of solid-state NMR (ssNMR)

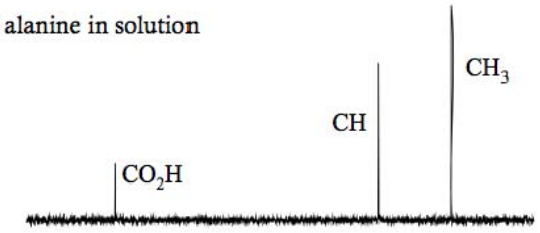


Outline

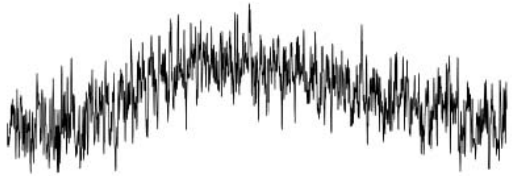
1. Anisotropic interactions
2. High-resolution ssNMR
   - Magic Angle Spinning (MAS) & Decoupling
   - Cross-Polarization
   - Recoupling techniques
3. Protein structure determination
   - Basics
   - Multidimensional ssNMR
   - Resonance assignment
   - Structural restraints
4. Applications

Anisotropic interactions

- ^{13}C NMR of alanine in solution



- ^{13}C NMR of solid alanine



NMR interactions

External ($\mathcal{H}_{ext}$) Internal ($\mathcal{H}_{int}$)

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_{RF} + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{CS} + \mathcal{H}_J$$

$\mathcal{H}_{ext} >> \mathcal{H}_{int}$ Magnitude

- $\mathcal{H}_z$: Zeeman interaction ~ 1000-100 MHz
- $\mathcal{H}_{RF}$: Interaction with RF pulse (B1) ~ 10-100 kHz
- $\mathcal{H}_Q$: Quadrupolar interaction (spin > 1/2 only !) ~ 10-100 kHz
- $\mathcal{H}_D$: Dipolar interaction ~ 10-100 kHz
- $\mathcal{H}_{CS}$: Chemical shift interaction ~ 10-200 ppm
- $\mathcal{H}_J$: Scalar interaction ~ 10-100 Hz

NMR interactions

Anisotropic Isotropic

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_{RF} + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{CS} + \mathcal{H}_J$$

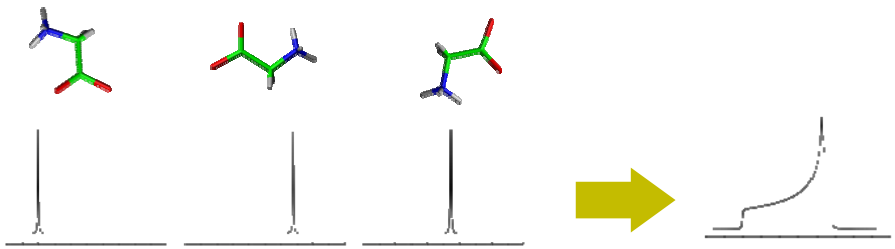
$\mathcal{H}_{aniso} >> \mathcal{H}_{iso}$ Magnitude

- $\mathcal{H}_z$: Zeeman interaction ~ 1000-100 MHz
- $\mathcal{H}_{RF}$: Interaction with RF pulse (B1) ~ 10-100 kHz
- $\mathcal{H}_Q$: Quadrupolar interaction (spin > 1/2 only !) ~ 10-100 kHz
- $\mathcal{H}_D$: Dipolar interaction ~ 10-100 kHz
- $\mathcal{H}_{CS}$: Chemical shift interaction ~ 10-200 ppm
- $\mathcal{H}_J$: Scalar interaction ~ 10-100 Hz

Anisotropic interactions

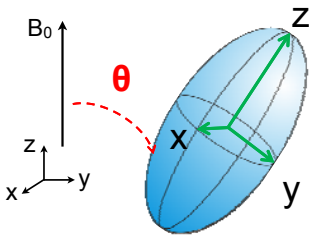
How do anisotropic interactions affect the NMR spectrum ?

- Each molecular orientation give rise to a different resonance frequency
- In powders we have a random distribution of molecular orientations. Thus the lineshape is the weighted superposition of all the different orientations



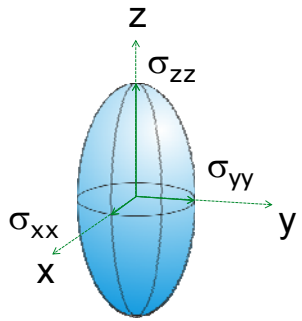
Anisotropic interactions

- Second-rank tensors (3 x 3 Matrices)



LAB

$$\sigma_{ISO} = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$$
$$\Omega = \sigma_{33} - \sigma_{11}$$
$$\kappa = \frac{\sigma_{ISO} - \sigma_{22}}{\Omega}$$
$$\sigma_{11} \le \sigma_{22} \le \sigma_{33}$$
$$\sigma_{11} + \sigma_{22} + \sigma_{33} \neq 0$$

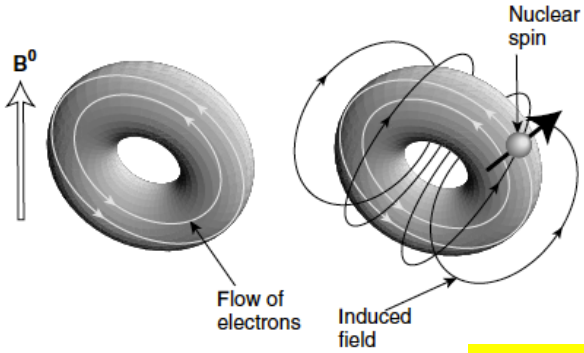


PAS

$$\sigma_{PAS} = \begin{pmatrix} \sigma_{11} & 0 & 0 \\ 0 & \sigma_{22} & 0 \\ 0 & 0 & \sigma_{33} \end{pmatrix}$$

Chemical Shift

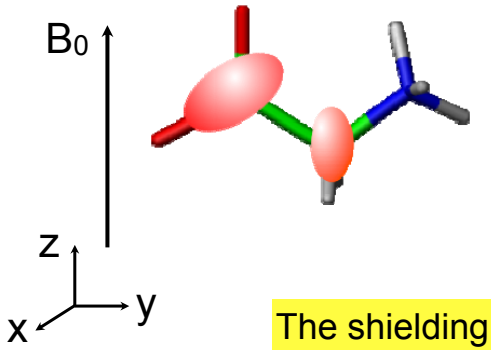
- The external magnetic field B_0 induces currents in the electron clouds in the molecule.
- The circulating molecular currents in turn generate a magnetic field (called the induced field $B_{induced}$):



Bj(loc)= B_0 + $B_{induced}$

Chemical Shift Anisotropy

- The electron distribution surrounding nuclei is inherently asymetric



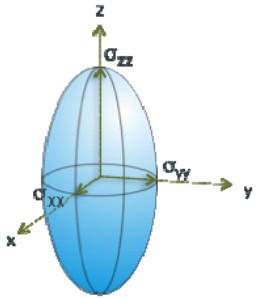
$$H_{CSA} = \sum_k \gamma_k \hat{I}_k \sigma_k \vec{B}_0$$

$$\begin{pmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{pmatrix}$$

The shielding effect depends :
- on the orientation with respect to B_0
- on the magnitude of B_0
- on the gyromagnetic ratio

Chemical Shift Anisotropy parameters

- By suitable choice of the coordinate system (PAS), the CSA Tensor only contains the diagonal elements



$$\sigma_{PAS} = \begin{pmatrix} \sigma_{11} & 0 & 0 \\ 0 & \sigma_{22} & 0 \\ 0 & 0 & \sigma_{33} \end{pmatrix}$$

with $\sigma_{11} + \sigma_{22} + \sigma_{33} \neq 0$

and $\sigma_{11} \le \sigma_{22} \le \sigma_{33}$

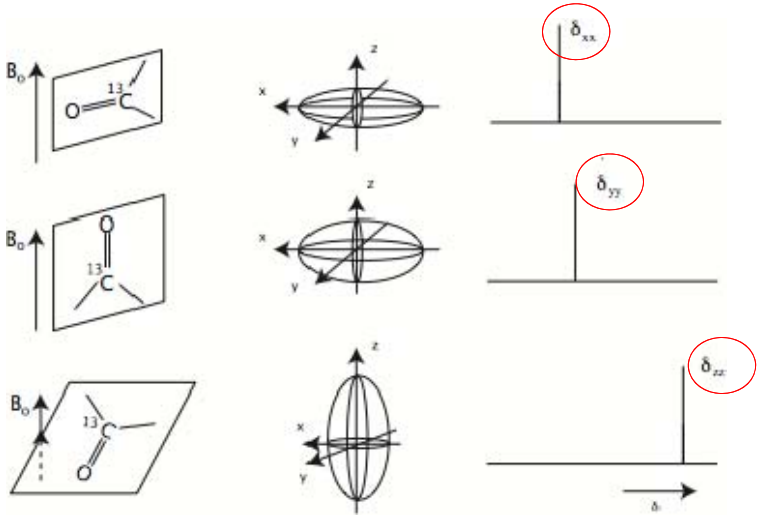
Isotropic chemical shielding $\sigma_{iso} = 1/3\ (\sigma_{11} + \sigma_{22} + \sigma_{33}) / 3$

Anisotropy (ppm) δ $\delta_{aniso} = \sigma_{33} - \sigma_{iso}$

Asymmetry $\eta_{asym} = (\sigma_{22} - \sigma_{11}) / \delta_{aniso}$

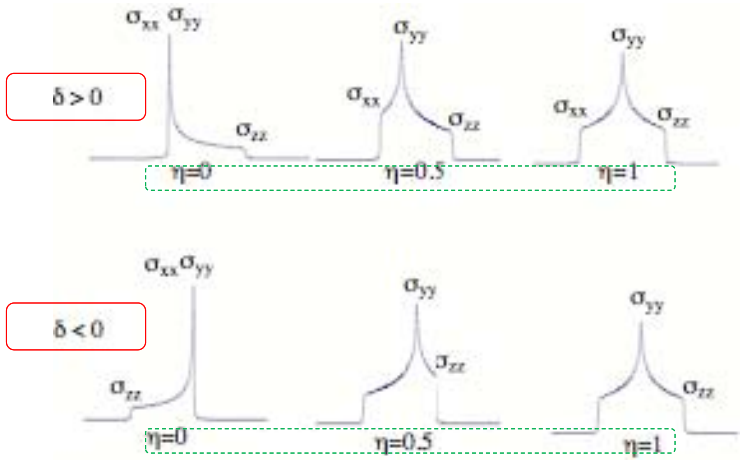
Chemical Shift Anisotropy

- The resonance frequency is directly correlated with the orientation of the CSA tensor with respect to B_0

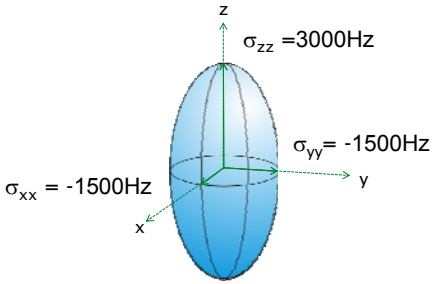


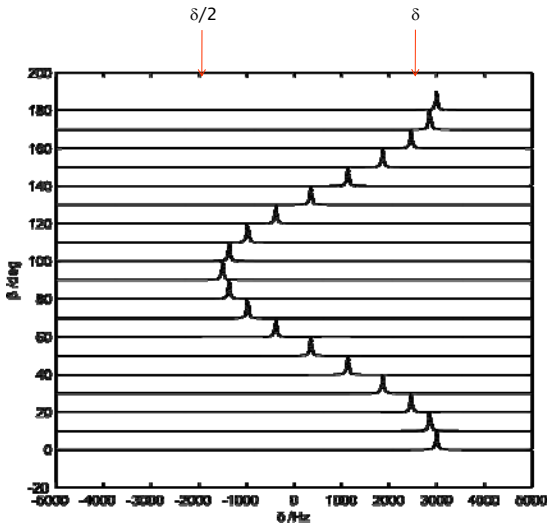
Chemical Shift Anisotropy

- Powder lineshapes are characteristic for the magnitude of the anisotropy (δ_{aniso}) and asymmetry (η_{asym}) of the CSA tensor



CSA for a single crystal





σ_{iso} = 1/3 (σ_{xx}+ σ_{yy}+ σ_{zz}) = 0 Hz

δ δ_{aniso} = σ_{zz} - σ_{iso} = 3000 Hz

η_{asym}= (σ_{yy}- σ_{xx}) / δ_{aniso}= 0.0

$$H_{CS} = \left(-\gamma_k B_0 \left(\sigma_{iso} + \frac{\delta}{2}\left[\left(3\cos^2\beta - 1\right) - \eta \sin^2\beta \cos 2\alpha\right]\right)\right)\hat{I}_{kz}$$

CSA for a single crystal

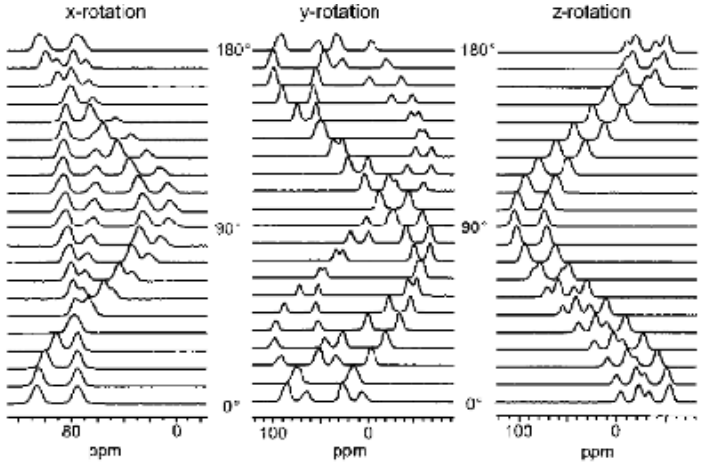
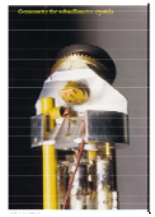
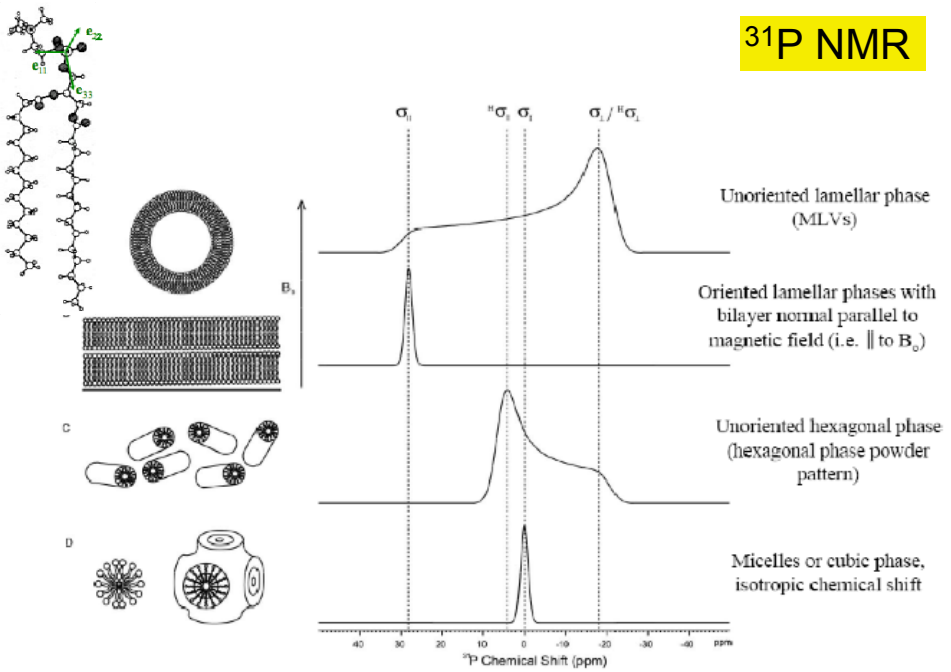


Figure 2. Phosphorus-31 CP NMR spectra of a single crystal of TMPS for rotations of the crystal holder about its *X*, *Y*, and *Z* axes, acquired at 4.7 T.

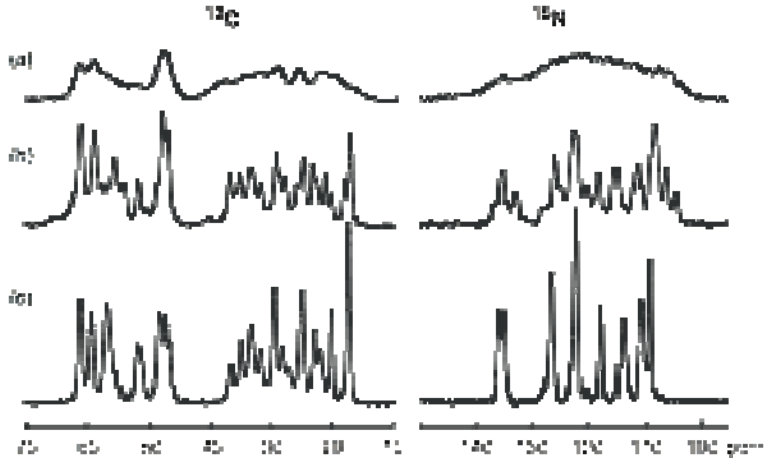


CSA characteristic for various lipid phases



Chemical Shift Anisotropy

- Lyophilized sample
- Microcrystalline powder
- Microcrystalline powder (different protocols)

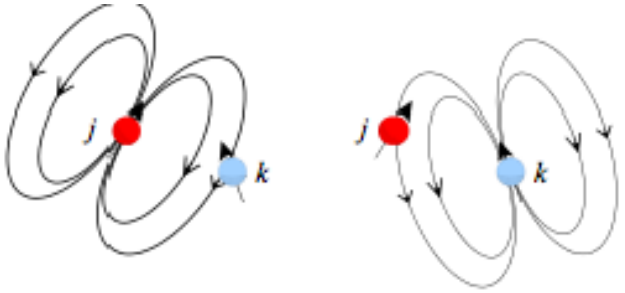


Chemical Shift Anisotropy

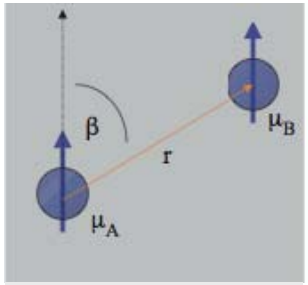
- Chemical shift (CS) depends on the local electronic environment
- CS can be calculated with quantum-mechanical methods and used to obtain structural information
- CS measurement are used to derive dihedral angles in protein backbone (Ca, C' atoms). The ^{13}C CS are characteristic of protein secondary structures

Dipolar coupling

= Interaction of one nuclear spin with a magnetic field generated by another nuclear spin, and vice versa



Dipolar couplings



Interaction energy between 2 magnetic (dipole) moments when both are aligned with B_0 :

$$E = \frac{\mu_0}{4\pi}\frac{1}{r_{AB}^3}\mu_A\mu_B(1-3\cos^2\beta)$$

Dipolar couplings depend on :
- orientation of the internuclear vector relative to the static field B_0
- internuclear distance ($1/r^3$)
- gyromagnetic ratios of the 2 involved nuclei

Dipolar couplings

Dipolar interaction tensor

$$D = \frac{\mu_0}{4\pi}\frac{\gamma_1\gamma_2\hbar}{r_{12}^3}\begin{pmatrix}1 & 0 & 0\\ 0 & 1 & 0\\ 0 & 0 & -2\end{pmatrix}$$

- Second-rank tensor
- Axially symmetric
- Traceless

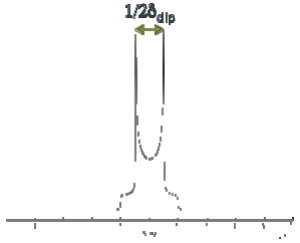
- In solution, dipolar couplings are averaged to zero due to rapid (rotational) motion of the molecules
- In solids, dipolar couplings give rise to line splittings which can easily exceed 10 kHz of magnitude.

Dipolar couplings

Heteronuclear dipolar coupling

$H_{IS} = -d\,(3\cos^2\beta - 1)\,I_z S_z$

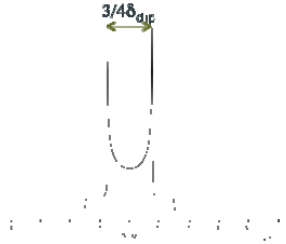
$d = (\mu_0/4\pi)\,\hbar\gamma_I\gamma_S/r_{IS}^3$



Homonuclear dipolar coupling

$H_{II} = -d/2\,(3\cos^2\beta - 1)\,3I_{1z}I_{2z} - I_1I_2$

$d = (\mu_0/4\pi)\,\hbar\gamma_I^2/r_{II}^3$



Nuclear pair	Internuclear distance	R^{DD} (Hz)
^{1}H- ^{1}H	10 A	120 kHz
^{1}H-^{13}C / ^{1}H-^{15}N	1 A	30 kHz
^{1}H, ^{13}C	2 A	3.8 kHz

Dipolar couplings

- Dipolar couplings are proportional to the internuclear distance : dipolar splittings in spectra of oriented or powder samples provide a high-precision measure of internuclear distances (CC, NC distances)
- Heteronuclear Dipolar couplings (~10-30 kHz) are used to obtain orientational restraints i.e. tilt of transmembrane helices in oriented bilayers (PISA wheels)
- Homonuclear ^{1}H-^{1}H dipolar couplings (up to 120 kHz) homogeneously broaden the NMR signals. Proton-detection in solids is difficult.

Motional averaging of anisotropic interactions

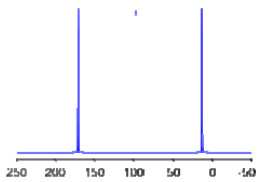
- Anisotropic interactions provide a rich source of information about amplitude motions in biomolecules
- They can possibly reveal details of the motions, such as asymmetric motions, as long as the process takes place on time scales shorter than the inverse of the anisotropic interaction strength e.g. for HN dipolar couplings (~10 kHz), motion amplitudes faster than 10 μs can be assessed
- The amplitude of the averaging process is reflected in the scaling factor of the anisotropic interactions and can be directly obtained from the lineshape analysis of ssNMR spectra

information about dynamics on different time scales

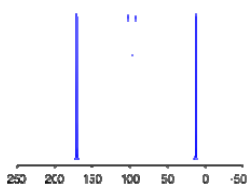
ssNMR spectrum (spin ½)

Isotropic

$H = H_{CS}$

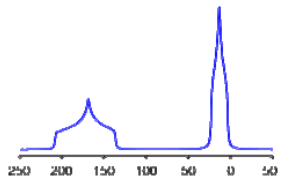


$H = H_{CS} + H_J$

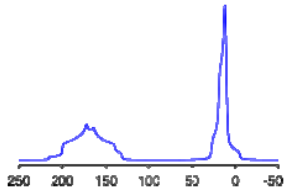


Anisotropic

$H = H_{CS} + H_J + H_{CSA}$



$H = H_{CS} + H_J + H_{CSA} + H_{Dipolar}$

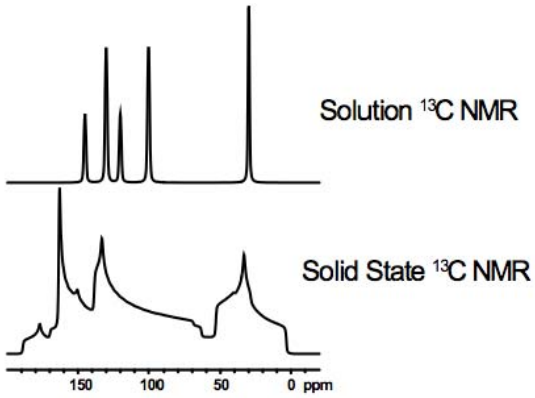


$H = H_{CS} + H_J + H_{CSA} + H_{Dipolar}$

ssNMR spectrum (spin ½)

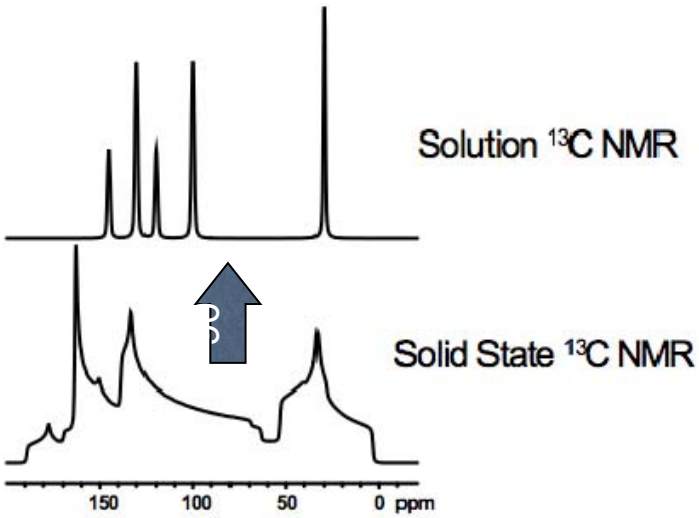
- Liquid: anisotropic interactions are averaged out on the NMR time scale. Only isotropic interactions are observable i.e. isotropic CS and scalar couplings
- Solid: NMR spectrum lineshape reflect the weighted sum of all orientations

Additional structural information



High-resolution ssNMR

NMR interactions

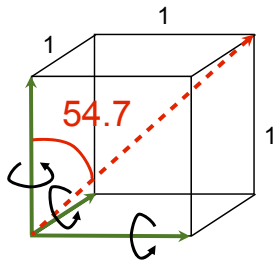


Magic Angle Spinning (MAS)

Andrew and Lowe (1954)

- MAS relates to sample spinning about an axis that is inclined by an angle θ to the static magnetic field B0

- If θ = 54.74° ('magic angle'), second-rank tensor interactions can be averaged out



Angular dependence of second-rank tensors is given by the second-order legendre polynomial $(3\cos^2\theta-1)/2$

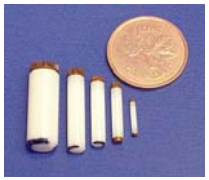
$(3\cos^2\theta-1)/2=0$ if $\theta=54.74°$

Magic Angle Spinning (MAS)

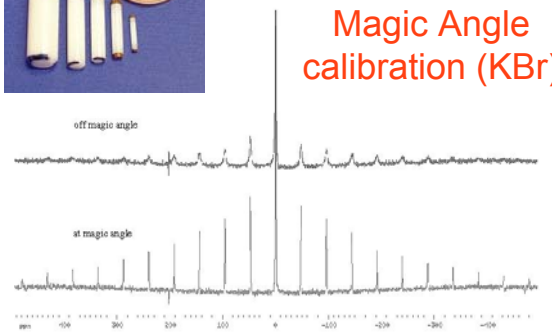
In MAS, the sample is placed in a cylindrical rotor which is rapidly spun about an axis that is tilted by 54.74 degrees away from the direction of the static B0.

MAS probe

MAS rotors



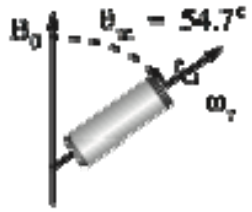
Magic Angle calibration (KBr)



Magic Angle Spinning (MAS)

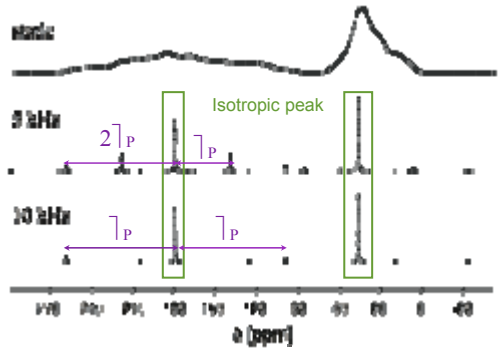
- MAS frequency > magnitude of the interaction : isotropic value

- MAS frequency < magnitude of the interaction : the powder spectrum is split into rotational sidebands

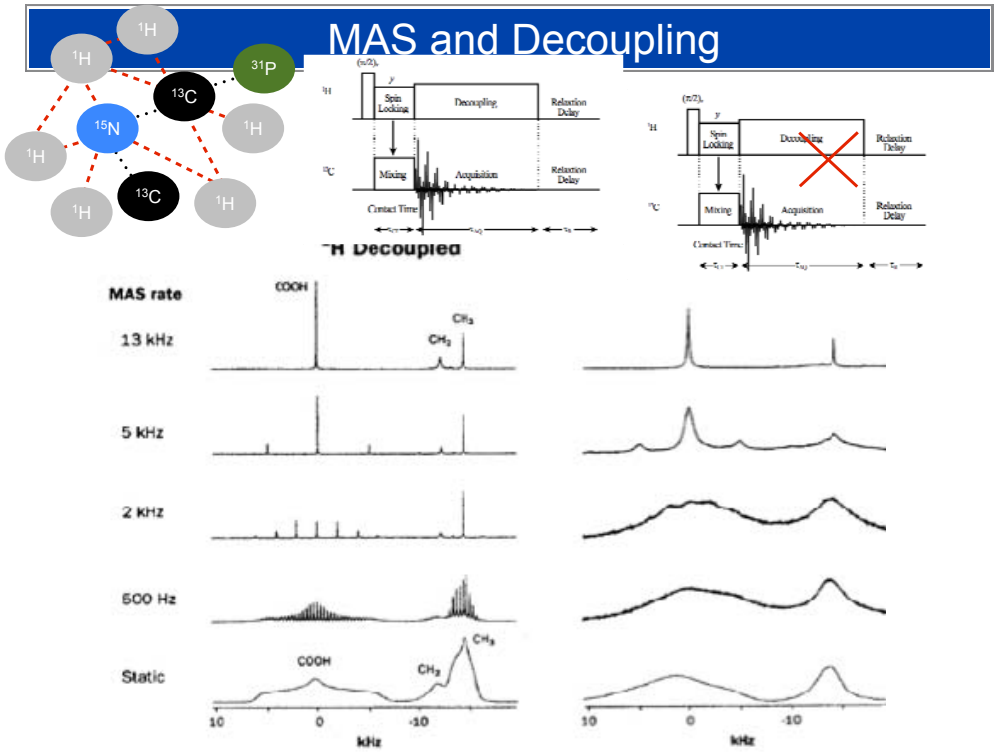


MAS speed (ν_P)

^{13}C spectra of Glycine (powder)



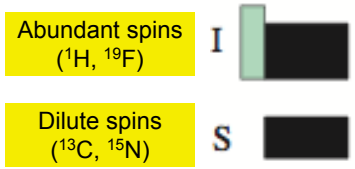
MAS and Decoupling



Cross Polarization (CP)

= Polarization transfer from abundant spins such as ^{1}H or ^{19}F to dilute spins such as ^{13}C or ^{15}N using through-space dipolar couplings. The overall effect is to enhance S/N.

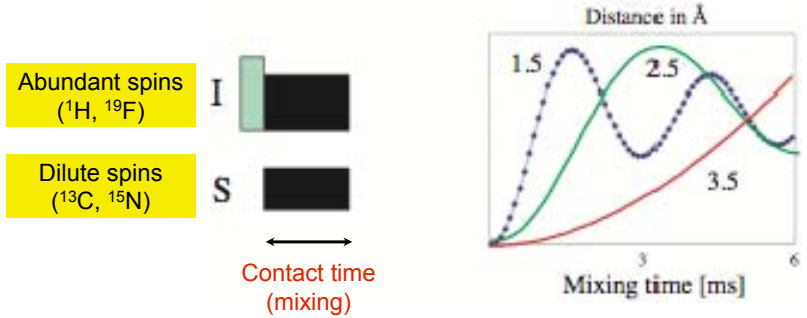
1. Signal enhancement factor (potentially) = γ_I/γ_S
2. Shorter recycling delay (T_1 ^{1}H << T_1 ^{13}C)



Cross Polarization (CP)

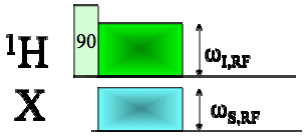
The extent by which magnetization can be transferred to the heteronucleus by CP depends on :

- the mixing time (or contact time)
- the magnitude of heteronuclear dipolar interactions (i.e. distance between the nuclei, dynamics)



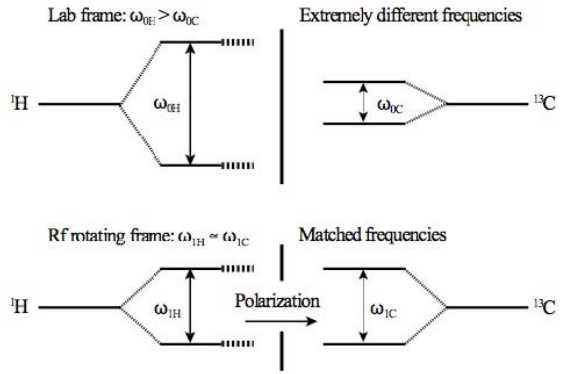
Cross Polarization (CP)

How to obtain efficient CP under MAS conditions



Hartmann-Hahn conditions :

$$\omega_{1I} - \omega_{1S} = \pm n.\omega_R \ (n = 1,2)$$



In the case of biological samples that exhibit a high degree of molecular mobility, CP can become ineffective ....

Recoupling techniques

Recoupling techniques utilize the constructive interference between rotations in real space (MAS) and rotations in spin space (RF irradiation). In this way, it is possible to avoid the averaging of anisotropic interactions by MAS

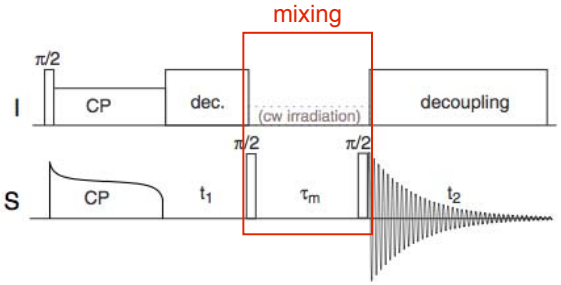
- Several classes of recoupling experiments :
  * without RF-irradiation
  * using discrete pulses
  * using CW irradiation
  * using phase-modulated RF irradiation

Obtaining structural restraints
Transfer the magnetization between nuclei

Recoupling techniques without RF irrdiation

1. Proton-Driven Spin Diffusion (PDSD)

= Dipolar-mediated polarization transfer under MAS (residual homonuclear ^{1}H-^{1}H dipolar couplings)

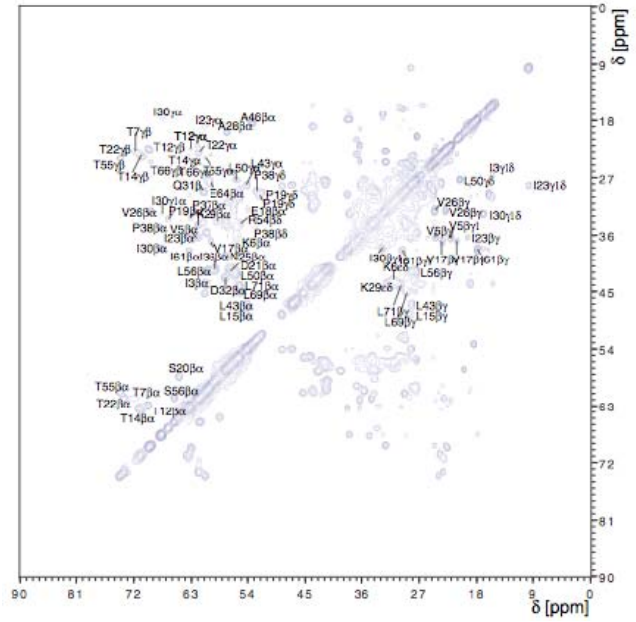


with CW ^{1}H irradiation at high MAS :

DARR: $\omega_{1H} = \omega_R$
HORROR : $\omega_{1H} = \omega_R/2$

Typical mixing times (protonated organic solids) :
- Direct bond transfer ~ 10 ms
- Long-range transfer ~ 100-500 ms

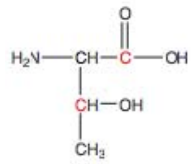
2D (^{13}C,^{13}C)-PDSD spectrum of Ubiquitin (mixing = 200 ms)



Recoupling techniques without RF irrdiation

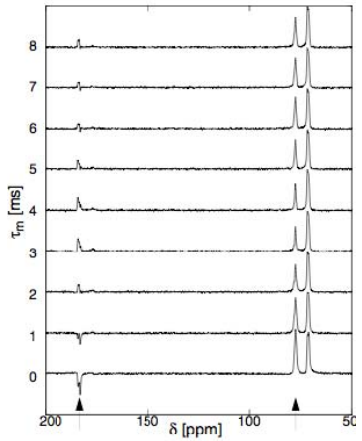
2. Rotational Resonance (R^2)

= " Rotor-driven" spin-diffusion



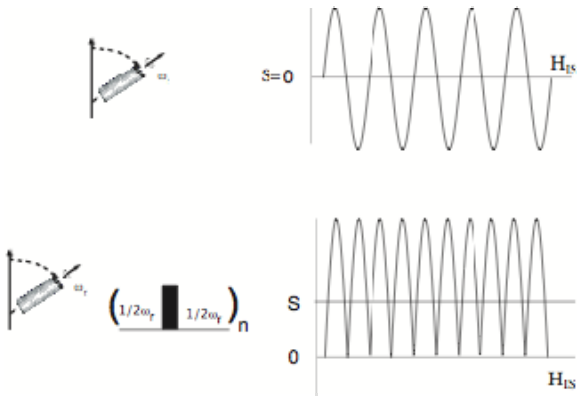
- Partial reintroduction of the homonuclear dipolar coupling when R^2 condition is fulfilled (line-broadening)
- Use as a polarization transfer method (PDSD under weak coupling conditions)

MAS frequency sets at
$\omega_R = \omega_1(CO) - \omega_1(C\alpha)$



Recoupling techniques with discrete RF-pulses

= Re-introduction of selected anisotropic spin interactions by applying rotor-synchronized RF pulses

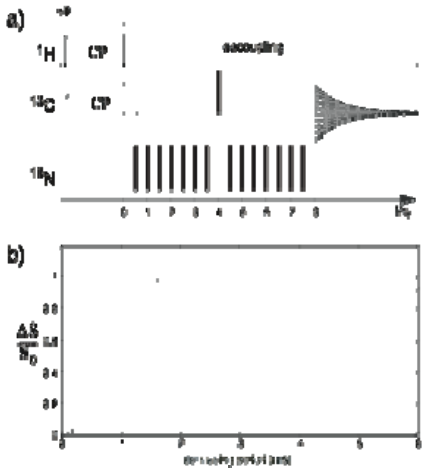


Discrete pulses prevent full averaging of the dipolar coupling under MAS !

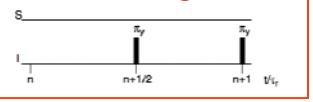
Protein structure determination and dynamics

Recoupling techniques with discrete RF-pulses

REDOR experiment



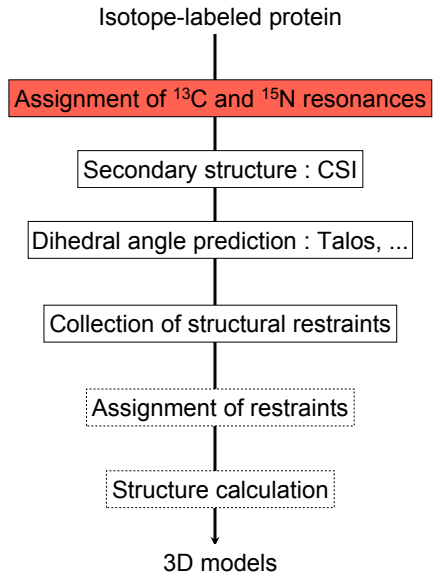
Basic building block

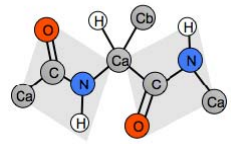


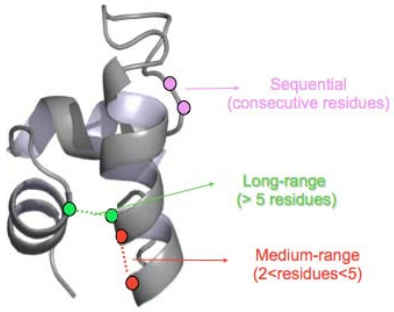
measurement of ^{13}C, ^{15}N heteronuclear dipolar couplings

Relative signal intensity change against mixing time for one-bond dipolar coupling

Structure determination protocol



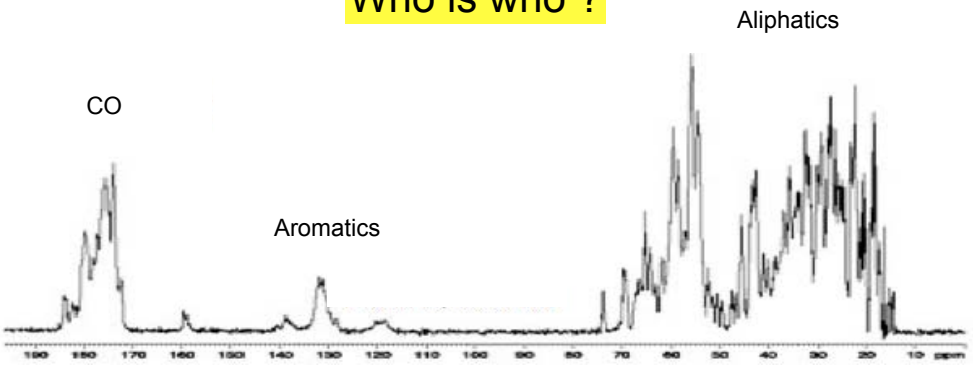




Resonance assignment

- Protein sequence known
- Chemical structure known

Who is who ?

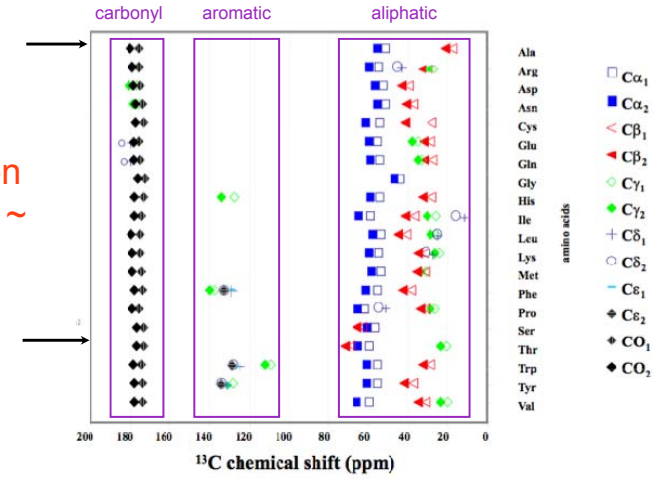


Resonance assignment

Averaged backbone and sidechain ^{13}C chemical shifts

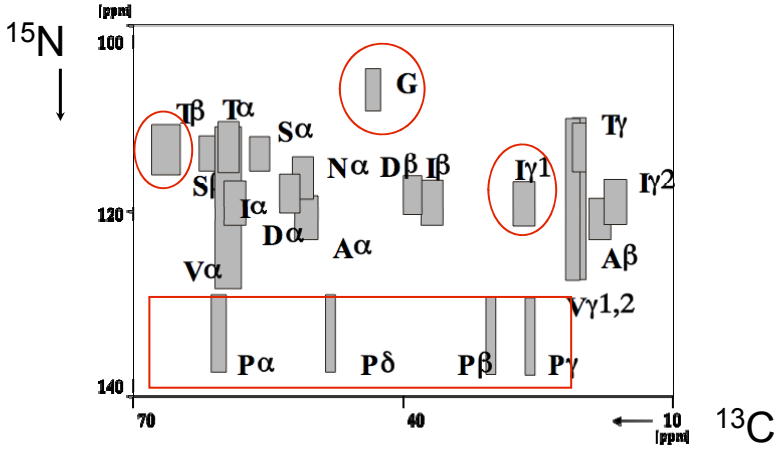
^{13}C shifts are ~ not amino-acid type specific

^{13}C-^{13}C correlation pattern is ~ unique !



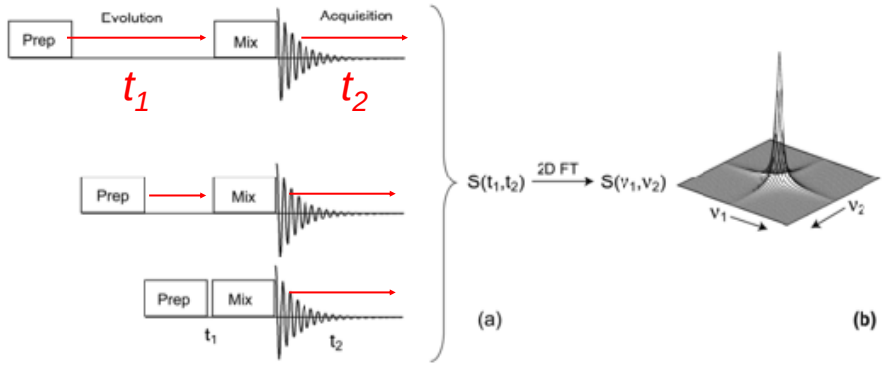
Resonance assignment

Characteristic ^{15}N-^{13}C correlations



Multidimensional ssNMR

- Evolution period is systematically incremented and a collection of FIDs $S(t_1,t_2)$ is recorded
- The 2D spectrum $S(\nu_1, \nu_2)$ is obtained by 2D FT of the $S(t_1,t_2)$
- Phase cycling of the preparation and mixing units selects the desired coherence transfer pathway



Multidimensional ssNMR

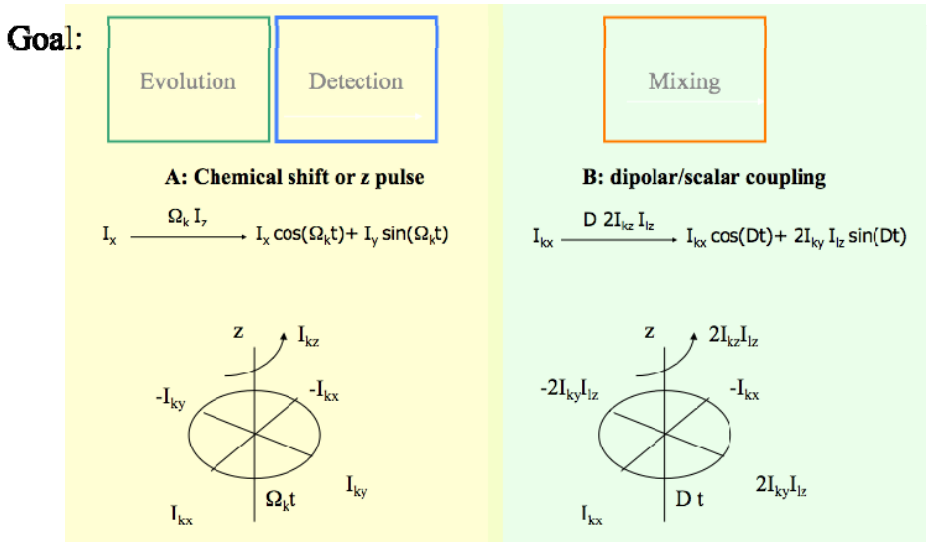
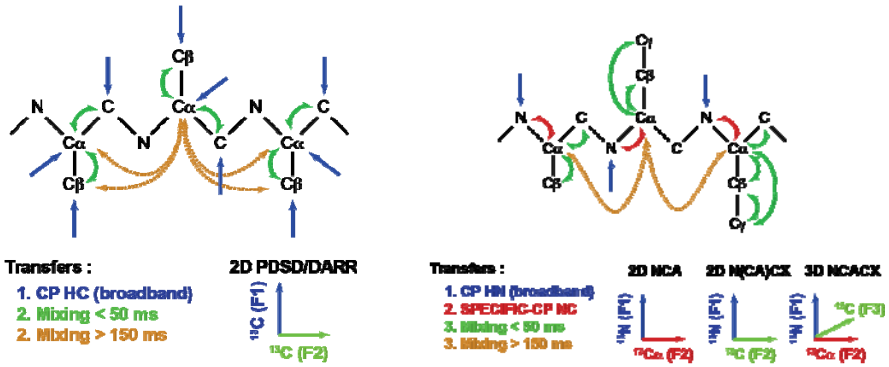


Fig.: Transformation properties of CS and dipolar/scalar mixing.

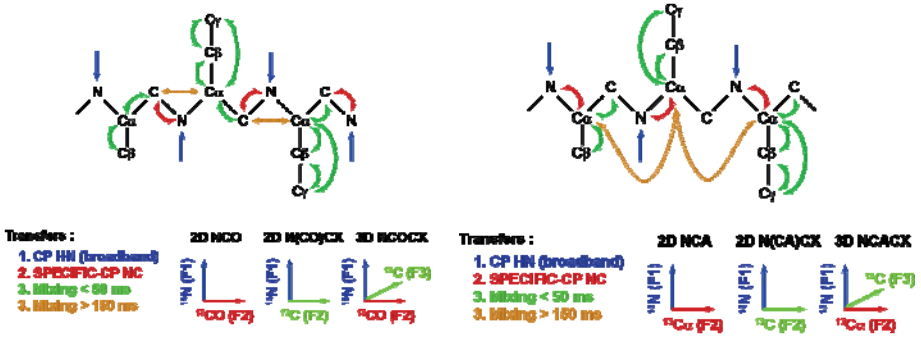
Sequential assignment of ^{15}N-^{13}C-labeled proteins

1. Identification of spin systems (amino-acid type) based on intraresidue correlation patterns
   - ^{13}C-^{13}C PDSD/DARR (short mixing time)
   - ^{15}N-^{13}C NCACX (short mixing time)
   - ^{15}N-^{13}C NCACB

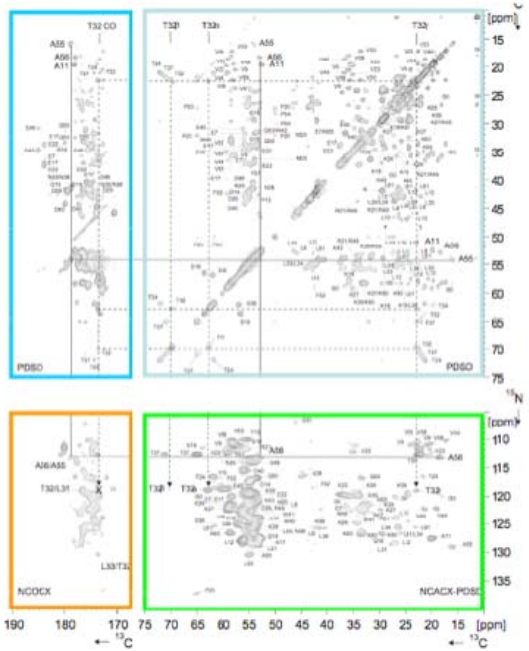


Sequential assignment of ^{15}N-^{13}C-labeled proteins

2. Linking the spin-systems by the identification of sequential correlations
   - ^{13}C-^{13}C PDSD/DARR (long mixing time)
   - ^{15}N-^{13}C NCACX (long mixing time)
   - ^{15}N-^{13}C NCOCX (short mixing time)
   - ^{15}N-^{13}C CANCO/CANCOCX



2D NC and CC correlation spectra



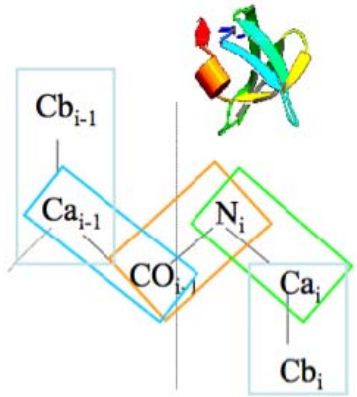
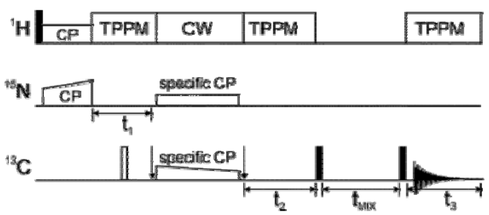
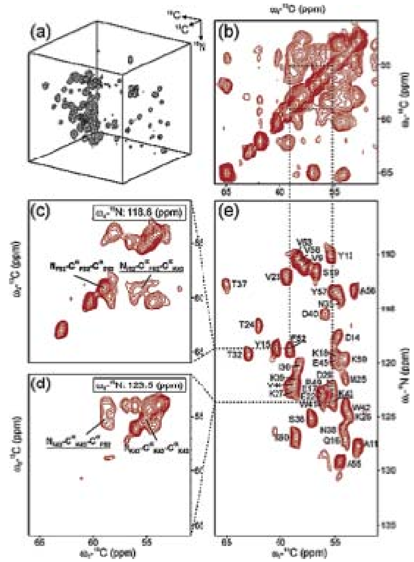


Figure Assignments in a 62 residue protein under MAS conditions

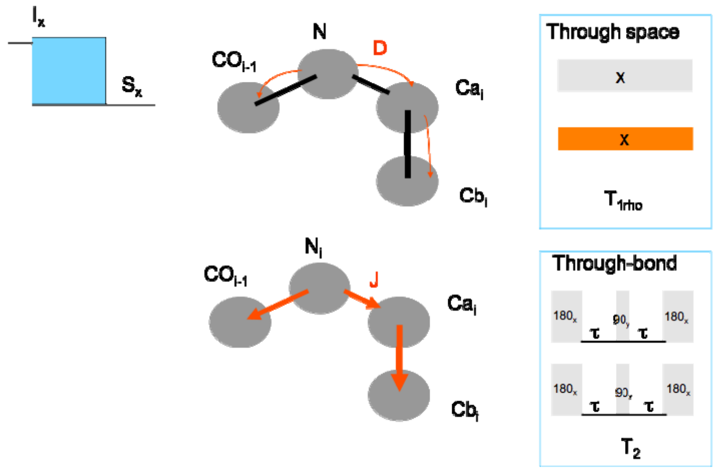
3D NCC correlation spectra





F. Castellani, B. J. van Rossum, A. Diehl, K. Rehbein, H. Oschkinat, Biochemistry 2003, 42, 11476.

Resonance assignment strategy

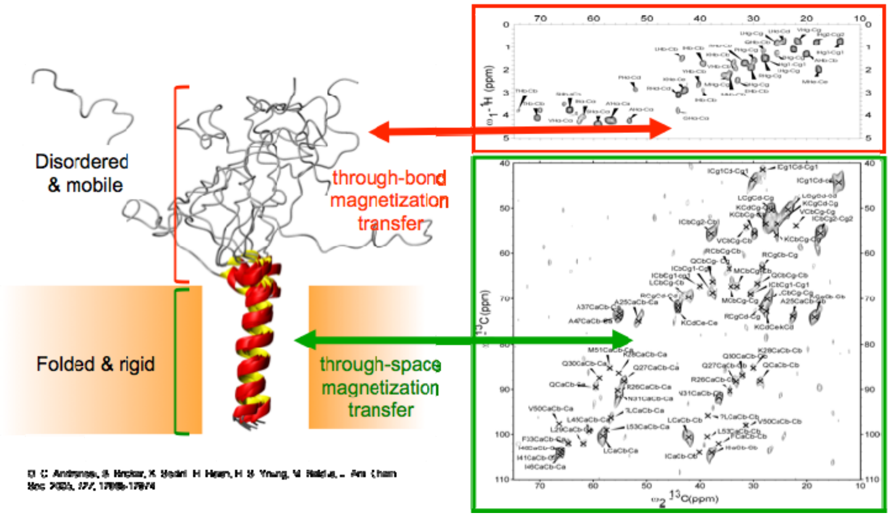


Rigid protein segments

Mobile protein segments

Sequential assignment of PLN in lipid vesicles

"Through-bond" *vs.* "through-space" transfer based ssNMR experiment



O. C. Andronesi, S. Becker, K. Seidel, H. Heise, H. S. Young, M. Baldus, J. Am. Chem. Soc. 2005, 127, 12965-12974

Structure determination protocol

Isotope-labeled protein

Assignment of ^{13}C and ^{15}N resonances

Secondary structure : CSI

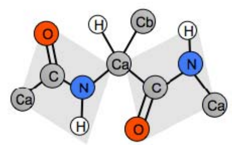
Dihedral angle prediction : Talos, ...

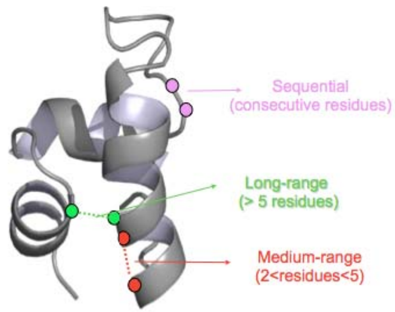
Collection of structural restraints

Assignment of restraints

Structure calculation

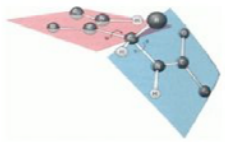
3D models



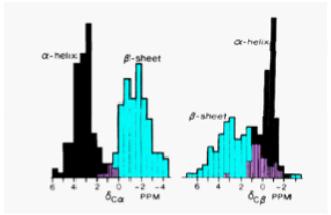


Secondary structure

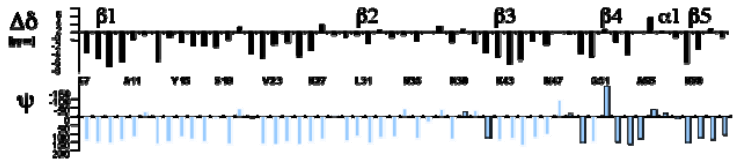
Dihedral angle prediction from ^{13}C and ^{15}N backbone CS (Talos)



Solution-state:



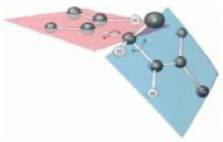
Solid-state:



Comparison of the backbone conformation in a 62 residue proteinand the secondary chemical shifts

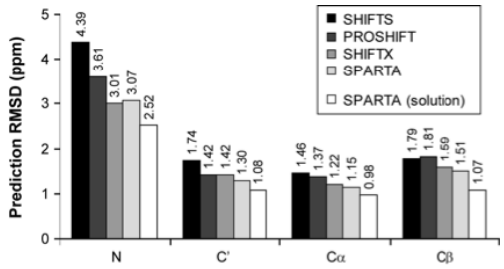
Luca S. D V Filippov J H van Boom, H Overkleeft, H J M de Groot and M Ottleo. Journal of Biomolecular Nmr. 2003 26(4) p 355-361

Secondary structure



^{13}C and ^{15}N backbone CS predictions from high-res 3D structures (ShiftX, ShiftX2,Sparta)

Molecule	Kind	Asgn. Res.	SSNMR source, year (BMRB entry)	Prediction source (PDB code, method)
BPTI	globular	9	McDermott et al. [23], 2000	Wlodawer et al. [24] (5PTI, X-ray)
Crh	globular (dimer)	63	Bockmann et al. [25], 2003	Joy et al. [26] (1MU4_A, X-ray)
GB1	globular	54	Franks et al. [27], 2005	Franks et al. [28] (2GI9, X-ray)
KcsA (Kv1.3)	membrane integral	72	Lange et al. [29], 2006; Schneider et al. [30]	Zhou et al. [27] (1K4C, X-ray), variation [29]
KTX	globular	26	Lange et al. [31], 2005 (6341)	Satoh et al. [32] (2KTX_1, soln. NMR)
LH2	membrane integral	75	van Gammeren et al. [21], 2005 (6348)	Papiz et al. [33] (1NKZ_D/E, X-ray)
MPX	membrane bound	12	Fujiwara et al. [34], 2004 (6214)	Todokoro et al. [35] (2C2P_1, ssNMR)
PLN (AFA)	membrane integral	29	Andronesi et al. [16], 2005	Andronesi et al. [16] (ssNMR)
SH3	globular	53	Paul et al. [36], 2001	Chevelkov et al. [37] (1U06, X-ray)
SRII	membrane integral	67	Etzkorn et al. [38], 2007	Royant et al. [39] (1H68, X-ray)
TRX	globular	103	Marulanda et al. [40], 2005	Katti et al. [41] (2TRX, X-ray)
TTR	fibril	9	Jaroniec et al. [42], 2004	Jaroniec et al. [42], 2004 (1RVS_1, ssNMR)
UBI	globular	63	Seidel et al. [43], 2005	Comilescu et al. [44] (1D3Z_1, soln. NMR)



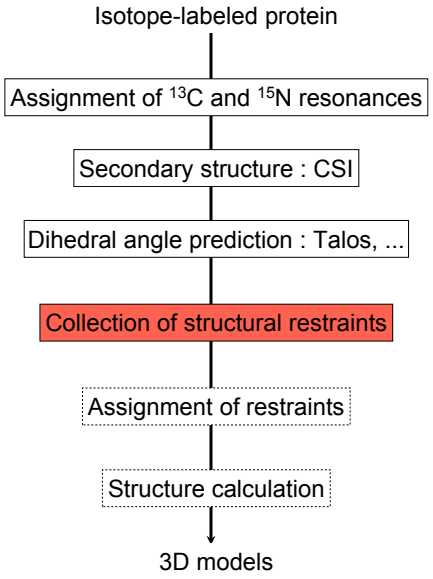
Saxena et al., Solu-state NMR, 2004

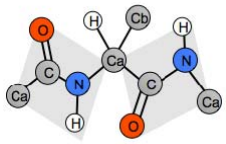
Tensor correlation experiments

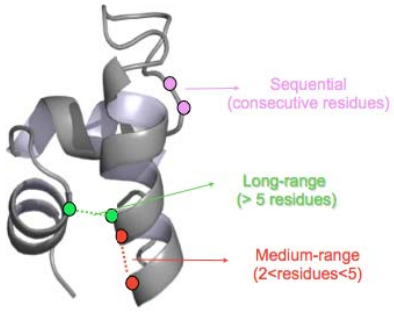
- Tensor correlations experiments can be used to determine the relative orientation of two anisotropic interactions

- For example, dihedral angles can be determined by :
  - Dipolar coupling tensor correlation
  - CSA / CSA tensor correlation
  - CSA / Dipolar tensor correlation

Structure determination protocol

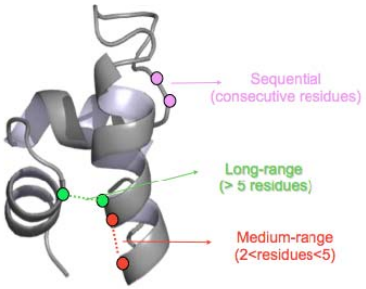






$^{13}C/^{15}N$ distance restraints

Measurement of internuclear distances by reintroducing the dipole-dipole coupling otherwise averaged out by MAS



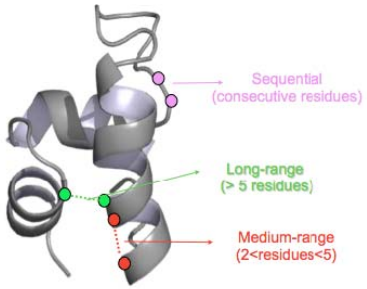
Accurate distances
but time-consuming !!

1. A single internuclear distance at a time :

- Specifically labeled spin-pair samples
- Frequency-selective recoupling methods

^{13}C-^{15}N distance restraints

Measurement of internuclear distances by reintroducing the dipole-dipole coupling otherwise averaged out by MAS



"Dipolar truncation effect" :
domination of strong dipolar couplings (i.e. short range), prohibiting the measurement of long-range distances

2. Hundreds internuclear distances at a time :

- Uniformly labeled samples
- Broadband recoupling methods

Distance restraints

PDSD is less sensitive to dipolar truncation

Protein Structure Determination from ^{13}C Spin-Diffusion Solid-State NMR Spectroscopy

Theofanis Manolikas,† Torsten Herrmann,‡ and Beat H. Meier*,†

Physical Chemistry, ETH Zurich, CH-8093 Zurich, Switzerland, and Institute of Molecular Biology and Biophysics, ETH Zurich, CH-8093 Zurich, Switzerland

Received October 19, 2007; E-mail: beme@ethz.ch

J|A|C|S ARTICLES

Published on Web 03/06/2008

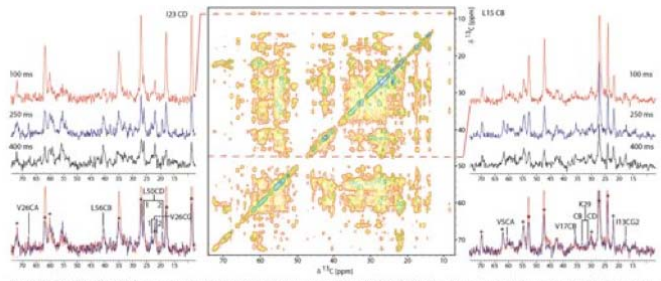
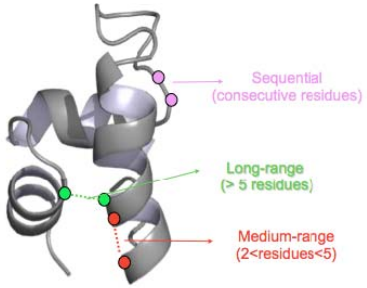


Figure 1. 2D $^{13}C-^{13}C$ PDSD spectrum of uniformly labeled ubiquitin recorded at 12 kHz MAS and a mixing time of 100 ms. The dashed lines indicate the region of one-dimensional (1D) slices extracted from 2D PDSD spectra at 100, 250, and 400 ms mixing time for I23CD (on the left) and L15CB (on the right). Beneath the 1D slices the overlay of the slices for 100 and 250 ms is given, indicating medium- and long-range correlations. Intraresidual and sequential contacts are marked with an asterisk.

$^{13}C,^{15}N$ distance restraints

Measurement of internuclear distances by reintroducing the dipole-dipole coupling otherwise averaged out by MAS



"Dipolar truncation effect" :
domination of strong dipolar couplings (i.e. short range), prohibiting the measurement of long-range distances

2. Hundreds internuclear distances at a time :

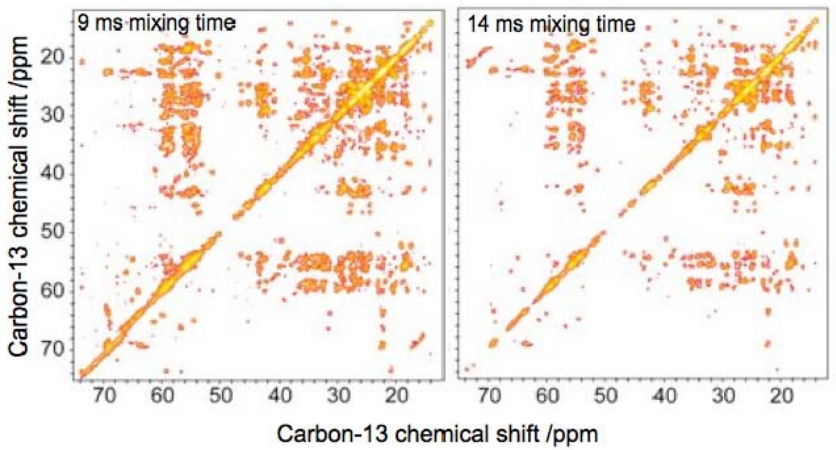
- Sparse isotope labeling schemes
- or Refined recoupling methods

Bayro MJ (2009), J.Chem. Phys 130, 114506

^{13}C,^{15}N distance restraints

Proton assisting the carbon spins

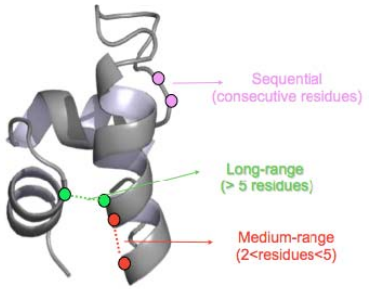
PAR spectra on microcrystalline CrH protein



De Paepe G, Lewandowski JR, Loquet A, Bockmann A, & Griffin RG (2008)
Proton assisted recoupling and protein structure determination. *J Chem Phys* 129(24):245101.

^{1}H-^{1}H distance restraints

Measurement of internuclear distances by reintroducing the dipole-dipole coupling otherwise averaged out by MAS



^{1}H-^{1}H distance restraints = most abundant source of inter-residue interactions in the range of up to 4 Å !

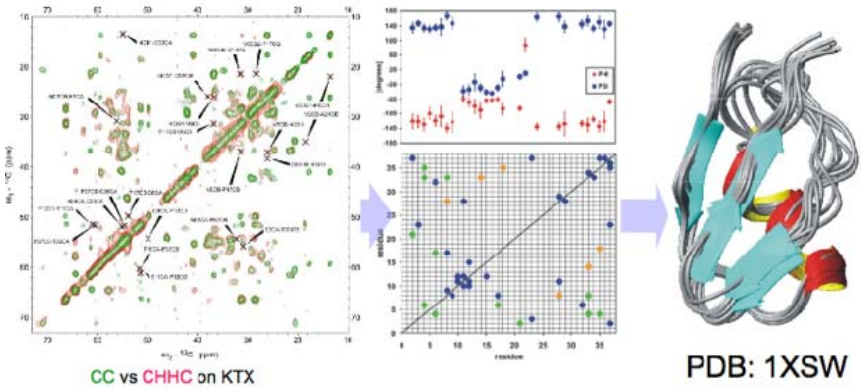
3. Hundreds ^{1}H-^{1}H internuclear distances at a time :

- Uniformly $^{15}N/^{13}C$ labeled sample
- Indirect detection schemes encoding ^{1}H-^{1}H polarization transfer

^{1}H-^{1}H distance restraints

CHHC and NHHC

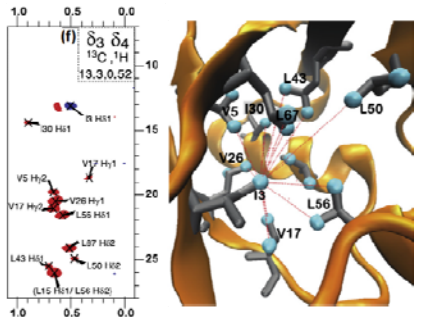
De novo solid-state NMR 3D structure of KTX



Backbone RMSD: 0.8 Å
Backbone RMSD (residues 4-38) between solid KTX and KTX in solution:1.9 Å

Lange A, Becker S, Seidel K, Pongs O, Baldus M. 2005. Angew. Chem.-Int. Edit. 44:2089.

^{1}H detection and ultra-fast MAS



Samoson A., Ernst M., Bockman A., Meier B. et al.
Angew. Chem. Int. Ed. 2014, 53, 12253 –12256

^{1}HN-^{1}HMe and ^{1}HN-^{1}HN distance restraints

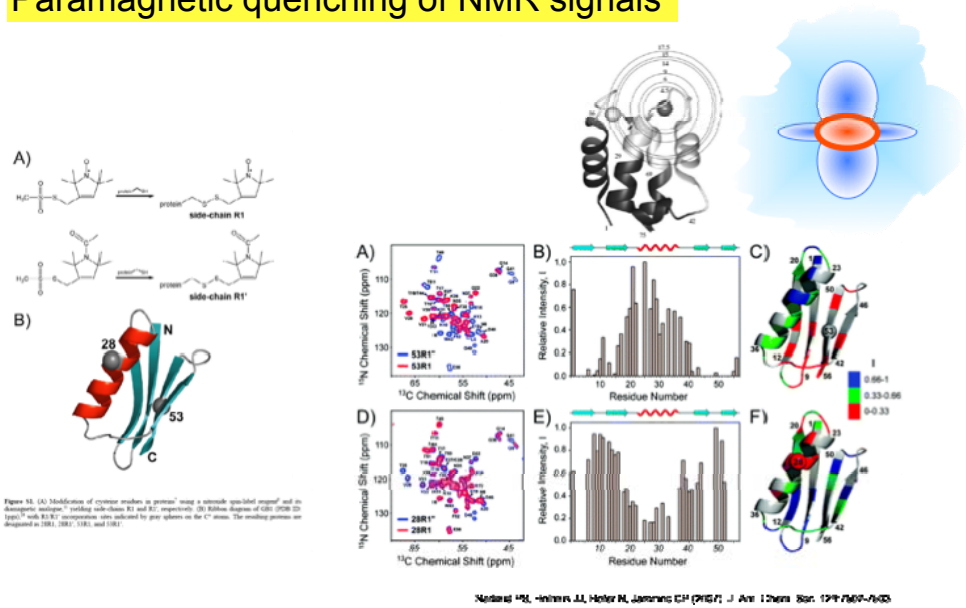
4D HSQC-DREAM-HSQC experiment

4. Specific ^{1}H-^{1}H internuclear distances :

- ^{1}H back-exchanged (U-$^{15}N/^{13}C/^{2}H$, ILV)-labeled sample
- Direct detection schemes encoding ^{1}H-^{1}H polarization transfer

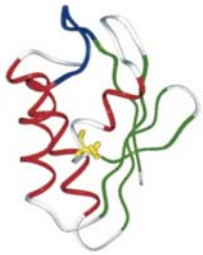
Other distance restraints

Paramagnetic quenching of NMR signals

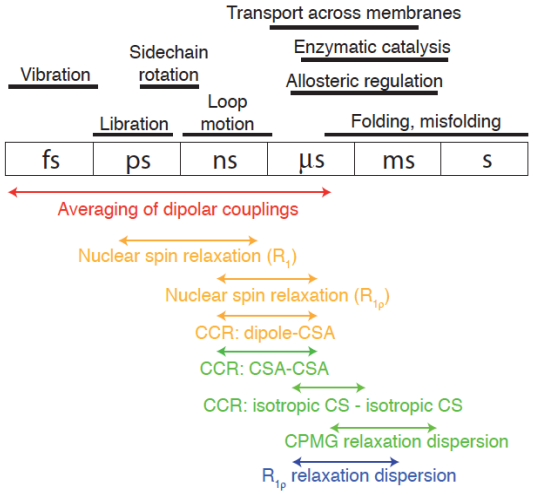


Protein dynamics on various timescales

3D structures and dynamics at the atom level



Protein fibrils
Membrane proteins
Weak protein assemblies
Etc.



Schanda, P. & Ernst, M. *Progress in Nuclear Magnetic Resonance Spectroscopy* **96,** 1–46

Biomolecular ssNMR : Summary

- No size limitations, crystallinity not mandatory
- Milligram amounts of ^{15}N-^{13}C (^{2}H) labeled protein
- Atom-level structure and dynamics in a (complex) functional environment
- About 70 protein structures (PDB/BMRB), including membrane proteins and fibrils
- Complex Biomolecules : spectral overlap and critical spectroscopic sensitivity

Resolution and Sensitivity Enhancement

- Refined isotope labeling strategies
- Multidimensional ssNMR experiments
- Efficient decoupling/recoupling schemes
- High static magnetic fields
- Proton-detection
- Fast (40 kHz) and ultra-fast MAS (111 kHz)
- DNP-MAS (400 MHz - 800 MHz)